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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: ID. No. 099101, 90 Day Inhalation Study in Rats with Benomyl

Tox. Chem. No.: 075A
Project No.: 2-1906
Record No. : S414984

FROM: Melba S. Morrow, D.V.M. MSM 6/9/92
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CONCLUSIONS:

Under the conditions of the study, benomyl, when administered to Crl:CDBR rats (Sprague Dawley) at concentrations of 0, 10, 50 and 200 mg/m^3 (0.96, 4.8, 19.2 mg/kg in males and 1.4, 7.0 and 28.8 mg/kg in females) was associated with olfactory degeneration at the highest dose tested in females and at 50 mg/m^3 in males. At 200 mg/m^3, males also had decreased body weights and body weight gains. The NOEL was 10 mg/m^3.

The mg/kg concentrations were derived using average body weights of 220 grams for females and 300 grams for males for the exposure period.

The study was classified as core supplementary based on the fact that there was no data on the range in particle size for the different concentrations tested and there was no data on the percent of the particles with aerodynamic diameters in the respirable range. Additionally, food consumption was not measured during the first 42 days of the test. Details of the particle size analysis are requested. The study may be upgraded upon receipt and evaluation of this data.

A copy of the DER is attached for your reference.

Printed on Recycled Paper

Reviewed by: Melba S. Morrow, D.V.M. 6/9/82 009549
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Joycelyn E. Stewart, Ph.D. 6/9/82
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: 90 Day Inhalation Study in Rats

GUIDELINE #: 82-4

TOX. CHEM. #: 75A

MRID #: 403995-01

TEST MATERIAL: Benomyl [redacted]

SYNONYMS: Benomyl, Methyl 1-(butyl carbamyl)-2-benzimidazolecarbamate 95.%

STUDY NUMBERS: 7986-001

SPONSOR: E. I. DuPont de Nemours
Newark, Delaware

TESTING FACILITY: Haskell Laboratories
Newark, Delaware

TITLE OF REPORT: Subchronic Inhalation Toxicity: 90 Day Study with Benomyl (INT-1991-484)

AUTHORS: David B. Warheit

REPORT ISSUED: August 10, 1987

CONCLUSIONS: Under the conditions of the test, the NOEL for benomyl, when administered to Sprague Dawley rats was 10 mg/m^3. The LOELs for males and females were 50 mg/m^3 and 200 mg/m^3, respectively based on the histologic observation of olfactory degeneration characterized by olfactory necrosis, chronic and acute inflammation, loss of olfactory epithelium and epithelial repair. At 200 mg/m^3 males also experienced decreased body weights and body weight gains. (Doses tested 0, 10, 50 and 200 mg/m^3 equivalent to 0.96, 4.8, and 19.2 mg/kg in males and 14, 7.0 and 28.8 mg/kg in females).

CLASSIFICATION: Core supplementary, based on the lack of information on the actual range in particle sizes and the percent of the particles with aerodynamic diameters less than 10 um for each concentration group and the lack of information on food consumption during the first 42 days of the study.

MATERIALS:

Carbamic acid [1-E(butylamino)carbonyl) - 1H-benzimidazol-2yl]-methyl ester, an off-white solid with a purity of 95.5% was the test material. Eighty male and 80 female Crl:CDBR rats were the test animals. The animals were obtained from Charles River Labs and at the start of the study, animals were 5 weeks of age and weighed from 89 to 175 grams.

METHODS:

During the 1 week quarantine period, animals were weighed and observed for clinical signs of disease. After the observation period, animals were randomly assigned to one of the following treatment groups:

Conc. (mg/m^3)	# Males	# Females
0	20	20
10	20	20
50	20	20
200	20	20

Animals were housed two to a cage during the study and were maintained in rooms with temperatures that ranged from 20-24^0 and relative humidity from 37 to 71%. Animals were maintained on a 12 hour light/dark cycle and food and water were available ad libitum, except during periods of exposure. These exposure periods were 6 hours a day, 5 days a week for approximately 14 weeks.

For control and high concentration groups, the 150 liter stainless steel chambers had an air flow rate of 60 liters per minute. Air flow in the low and mid concentration groups was 75 and 150 liters per minute, respectively. All chambers were equipped with nose cones and animals were rotated within the chamber during each exposure to ensure that no animal was located in the same position during two consecutive exposures. This was done because there was a potential for variation in the chamber concentration.

Atmospheres of benomyl were generated with bin feeders that were equipped with twin stainless steel feed screws. Benomyl dust was metered into a polycarbonate transfer tube. Test material was swept into exposure chambers by high pressures of air. Benomyl concentrations in the chamber were controlled by the rate that the dust was fed into the generation apparatus or by changing the air flow rate.

Samples of benomyl in the chambers were collected from the breathing zone at approximately 50 minute intervals. Sample were collected by drawing volumes of chamber atmosphere through the preweighed glass filters. Filters were weighed and atmospheric concentrations were determined from filter weight differences recorded pre and post sampling.

Particle size was determined with the Sierra Cascade impactor and was reported as mass median aerodynamic diameters and as the percent of particles with aerodynamic diameters of less than 10 microns.

Temperature in the chambers was measured hourly during the 6 hour period of exposure. Chamber oxygen concentrations were measured once per exposure with Biosystems Model 3100R Oxygen Analyzer.

Animals were weighed weekly and were observed daily for clinical signs of toxicity or disease. Food consumption was also determined weekly. Ophthalmoscopic examinations were conducted prior to the start of the study, at the study's midpoint (45 days) and at the end of the study. Ten males and ten females were selected for evaluation of clinical pathology parameters on days 45 and 90. An additional 9 females were selected from the 10 mg/m^3 exposure group for clinical pathology evaluation at day 45 and an additional 10 females were selected from the same group for evaluation at 90 days.

Blood was collected from the orbital sinus of each animal to evaluate the following:

x Hematocrit (HCT)
x Hemaglobin (HGB)
x Leukocyte count (WBC)
x Erythrocyte count (RBC)
x Platelet count
x Leukocyte differential
x Mean corpuscular hemaglobin
x Mean corpuscular hemaglobin concentration
x Mean corpuscular volume
Reticulocytes
Blood clotting measurements:
Thromboplastin time
Clotting time
Prothrombin time

Electrolytes:
x Calcium
Chlorine
Magnesium
Phosphorous
x Potassium
x Sodium

Enzymes:
x Creatinine phophokinase
x Alkaline phosphatase
x Lactic dehydrogenase
x SGPT (ALT)
x SGOT (AST)
Gamma glutamyl transferase
Glutamate dehydrogenase
Cholinesterase

Other Serum Chemistry Values:
x Albumen
x Blood creatinine
x BUN
x Cholesterol
x Globulin
x Glucose
Total Bilirubin
x Total protein
Triglycerides
Serum protein electrophoresis

A full gross necropsy was performed on ten animals per sex per group (9 for group 4) after day 45. The remaining animals were sacrificed after 90 days of exposure. Animals were sacrificed while they were under cloroform anesthesia by exsanguination.

Lungs were weighed and were infused with a fixative.

The following CHECKED (x) tissues were collected for histological examination from control and high concentration groups and any animal dying in extremis. Only the nasal cavity, lungs, liver, kidneys , testes and gross lesions were examined for animals in teh other groups. Checked (xx) tissues were also weighed.

Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	x Aorta	xx Brain
x Salivary glands	xx Heart	x Periph. nerves
x Esophagus	x Bone marrow	x Spinal cord
x Stomach	x Lymph nodes	
x Duodenum	xx Spleen	
x Jejunum	x Thymus	Glandular
x Ileum		x Parathyroids
x Cecum		x Adrenals
x Colon	Urogenital	x Thyroid
x Rectum	xx Kidneys	x Pituitary
	x Urinary bladder	x Mammary
xx Liver	xx Testes	x Harderian
Gall bladder	x Epididymides	Other
x Pancreas	x Prostate	Bone
	x Seminal vesicle	x Skin
Respiratory	x Ovaries	x Skel. muscle
x Trachea	x Uterus	x All gross lesions
x Lung	x Vagina	x Eyes/lacrimal gl.
x Nasal cavity	x Cervix	
Pharynx		
Larynx		

QUALITY ASSURANCE: A statement of quality assurance was included in the submission. The statement was dated August 10, 1987.

RESULTS:

Chamber Atmosphere Analysis

Mean particle sizes ranged from 1.7 microns to 2.0 microns. In the exposure chambers, the temperature ranged from 24- 26° C and the relative humidity ranged from 39 to 46%. (See Table I)

Mortality

No treatment related deaths were reported during the study. One female in the high dose group escaped on day 10 of exposure and was found on in poor condition. On day 13 of the study, this animal was sacrificed and was not subjected to gross or microscopic examination.

Body Weight, Weight Gains and Food Consumption

In the animals receiving high concentrations (200 mg/m^3) a significant decrease in body weight gains was reported in males during the first 37 days of exposure. This decrement was 13.5% lower than the weight gain reported for controls. During the interval which included days 64 through 92, mean body weight gains were significantly lower in all exposed females. Body weight gains in these animals were 27, 35.5 and 48 percent lower than controls for low, mid and high exposure groups, respectively. In low dose males, body weight gain was significantly increased over that of control males at the interval which included days 64 -92.

In the male high exposure group, significant decreases in body weight were reported on days 37, 42 and 57 through 85. Body weight decrements ranged from 6.1 to 10.8 percent. At various intervals throughout the study, significant differences in body weights of males in the high concentration group were reported. (See Table II).

Reduced food consumption was also reported for males in the high concentration group. Feed efficiency for the duration of the study could not be calculated because measurements of food consumption were not recorded until day 42 of the study.

Clinical Observations, Gross Pathology and Histopathology

Clinically, there were incidences of stained abdomen and ears and alopecia reported in the high exposure group. These findings were considered incidental and lacked biological significance. Alopecia was slight and affected only 3 females and varied in anatomical location. Staining was reported in 2 females, one of which also had slight alopecia. These clinical signs were not observed in males.

With regard to hematology and serum chemistry parameters, none of the observations could be associated with the administration of the test material. At the 45 day interval, no significant observations were made in males. Females in the low dose group had nonsignificant elevations in MCV, MCH and MCHC; females in the mid dose group had elevated MCHC, MCH and lymphocytes and in the high dose group, nonsignificant increases were reported in MCHC and MCH and decreases were reported in the number of neutrophils. By 90 days, these changes were not present in females. In high dose males at 90 days, an increase in the number of atypical lymphocytes was reported and decreases in the hematocrit and the number of monocytes were reported.

At 45 days, glucose was elevated in low concentration females and mid concentration males, creatinine was decreased in all groups of males and potassium was decreased in intermediate and high concentration males. At 90 days, glucose was normal in both sexes and AST, total protein and albumen were decreased in high concentration females. In the high concentration males, creatinine, globulin, sodium and potassium were decreased. Sodium was also decreased in low concentration males and creatinine was decreased in males receiving benomyl at intermediate concentrations. None of the reported findings were significantly different than controls.

There were no changes in the urinalysis data that could be associated with the administration of benomyl.

Pathology

No treatment related changes were reported for organ weights. Additionally, there were no gross lesions that were associated with the administration of the test material. Histologically, degeneration of the olfactory epithelium was reported after 45 days in all males and 8/10 females exposed to benomyl at concentrations of 200 mg/m^3. At 50 mg/m^3, two of the ten rats evaluated had mild olfactory degeneration.

After 90 days on the test, all rats exposed to the highest concentrations of benomyl had olfactory degeneration and 3/10 males exposed to the intermediate concentration had lesions which also suggested olfactory degeneration.

Incidences of inflammation of the respiratory epithelium were reported in all groups, including the controls. Respiratory inflammation was not related to benomyl since it occurred with similar frequency in control and treated groups.

In two males in the high concentration group, unilateral spermatid depletion was observed. It was noted in the report that in these two animals, spermatogenesis was unaffected, as was the sperm maturation process. The observed unilateral spermatid depletion was not considered to be related to the administration of benomyl.

DISCUSSION:

Based on the results of this study, the NOEL was 10 mg/m^3. The LOEL was 50 mg/m^3 in males and 200 mg/m^3 in females based on the histological observation of degeneration of the olfactory epithelium which involved necrosis of the olfactory epithelium, acute and chronic inflammation, loss of olfactory epithelium and epithelial repair. In male rats receiving 200 mg/m^3, there was also decrements in body weights and body weight gains during the first 37 days on the study.

The variations observed in the clinical chemistry parameters were not considered to be related to the administration of benomyl because they were sporadic in occurence and for the most part were within the acceptable normal value range.

Benomyl has been associated with decreased spermatogenesis at doses above 7.5 mg.kg (based on acute rat inhalation study, MRID # 00097281). It is believed that the unilateral effects on spermatids observed in two males receiving high concentrations of benomyl are not related to the administration of the test material because systemic exposure should not result in a unilateral effect. Additionally, there was no effect on the sperm maturation process as has been demonstrated in earlier studies with benomyl.

The study is classified as core supplementary based on the fact that no information was provided on the range in particle sizes nor was there data provided on the percent of particles with aerodynamic diameters less than 10 um. In addition, food consumption data were not available for the duration of the study. Once details of particle size are received, the study may be upgraded.

TABLE I
CHAMBER ATMOSPHERE

Target Conc.	Mean measured Concentration	Concentration Range	Mean Particle size
0	0		
10	10.7	3.3 - 23.7	1.7
50	49.7	20.0 - 122.0	1.9
200	199.0	2.0 - 541.0	2.0

TABLE II
MEAN BODY WEIGHTS (g) in MALES

Days on Test	Body weights	
	0 mg/m^3	200 mg/m^3
2	161.4	161.6
21	283.4	279.3
37	364.2	337.3*
42	382.1	359.0*
57	437.8	394.8*
64	455.9	408.1*
71	471.4	420.5*
78	473.5	429.4*
85	484.4	440.4*

* = $p < 0.05$

No differences were observed at 10 and 50 mg/m^3
(Table extracted from Table I of report)

No statistically significant differences in body weights were recorded for females in any of the exposure groups.